

PCTA9407-2(한국생명공학연구원)

<110> Korea Research Institute of Bioscience and Biotechnology

<120> A novel *Hansenula polymorpha* gene coding for alpha 1,6
mannosyltransferase and process for the production of recombinant
glycoproteins with *Hansenula polymorpha* mutant strain deficient
in the same gene

<160> 14

<170> KopatentIn 1.71

<210> 1

<211> 1351

<212> DNA

<213> *Hansenula polymorpha*

<220>

<221> CDS

<222> (10)...(1293)

<400> 1

cgggtgaaga	atg	gtg	tat	ttt	tta	aat	ttc	atg	tca	ata	acc	aat	gtc	ccg	51
	Met	Val	Tyr	Phe	Leu	Asn	Phe	Met	Ser	Ile	Thr	Asn	Val	Pro	
	1	5		10											

gtg	ctg	aag	cgc	gcf	cga	ctc	tac	atg	gcf	acg	aat	cgc	ccg	ctg	gtg	99
Val	Leu	Lys	Arg	Ala	Arg	Leu	Tyr	Met	Ala	Thr	Asn	Arg	Arg	Leu	Val	
15	20		25			30										

gtt	gtt	ctt	gtg	gtg	ctg	ctg	tac	tgg	gtg	gtc	cag	aac	gtt	tgg	acg	147
Val	Val	Leu	Val	Val	Leu	Leu	Tyr	Trp	Val	Val	Gln	Asn	Val	Trp	Thr	
35		40			45											

tgg	agc	cct	ggg	acg	cgc	gat	ttg	gcc	caa	gtg	gac	gcf	aag	atc	gag	195
Trp	Ser	Pro	Gly	Thr	Arg	Asp	Leu	Ala	Gln	Val	Asp	Ala	Lys	Ile	Glu	
50		55		60												

gcc	gag	cta	aac	tcg	aat	cta	cat	act	ttt	gga	gcf	cat	ttg	cgc	cac	243
Ala	Glu	Leu	Asn	Ser	Asn	Leu	His	Thr	Phe	Gly	Ala	His	Leu	Arg	His	
65		70		75												

tta	aac	cgf	ctt	ccg	gca	gag	tcg	gcc	acc	ctg	cgt	gaa	aaa	ctc	acc	291
Leu	Asn	Arg	Leu	Pro	Ala	Glu	Ser	Ala	Thr	Leu	Arg	Glu	Lys	Leu	Thr	
80		85		90												

ttc	tat	ttc	cca	tat	tat	cct	gaa	aag	ccc	gtg	ccg	aac	cag	atc	tgg	339
Phe	Tyr	Phe	Pro	Tyr	Tyr	Pro	Glu	Lys	Pro	Val	Pro	Asn	Gln	Ile	Trp	
95		100			105		110									

cag	aca	tgg	aag	gtc	gat	ctc	gaa	gac	gac	aac	ttc	ccc	aag	cag	tac	387
Gln	Thr	Trp	Lys	Val	Asp	Leu	Glu	Asp	Asp	Asn	Phe	Pro	Lys	Gln	Tyr	
115		120			125											

aga	cgg	ttt	cag	aag	acg	tgg	gtc	gag	aaa	aat	cca	gac	tac	gtg	tac	435
Arg	Arg	Phe	Gln	Lys	Thr	Trp	Val	Gl	Lys	Asn	Pro	Asp	Tyr	Val	Tyr	
130		135			140											

cac	ctg	att	ccg	gac	tct	gtg	att	gag	gac	ttt	gtg	ccg	agt	ttg	tac	483
His	Leu	Ile	Pro	Asp	Ser	Val	Ile	Glu	Asp	Phe	Val	Ala	Ser	Leu	Tyr	
145		150			155											

gcg	aac	gtg	ccg	gag	gtg	gtc	aga	gcf	tac	cag	ctg	ctt	ccg	aaa	aat	531
Ala	Asn	Val	Pro	Glu	Val	Val	Arg	Ala	Tyr	Gln	Leu	Leu	Pro	Lys	Asn	
160		165			170											

atc	atg	aag	gcf	gat	ttt	ttc	ccg	tat	ttg	gtg	atc	tac	gcf	ccg	gga	579
Ile	Met	Lys	Ala	Asp	Phe	Phe	Arg	Tyr	Leu	Val	Ile	Tyr	Ala	Arg	Gly	
175		180			185			190								

ggc	acc	tac	tca	gac	atg	gac	acg	gtg	tgt	tta	aag	ccg	atc	aag	gac	627
Gly	Thr	Ser	Asp	Met	Asp	Thr	Val	Cys	Leu	Lys	Pro	Ile	Lys	Asp		
195			200			205										

tgg	gcc	acg	ttt	gat	ccg	gac	atc	cac	gct	gcc	gac	aat	aag	gcc	Trp	675
Ala	Ala	Thr	Phe	Asp	Arg	Asp	Leu	Ile	His	Ala	Ala	Asp	Asn	Lys	Ala	

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210	215	220	
gat ctc tcc cag ata gat cca gaa gca aga acc acg cct	gag	acc	723
Asp Leu Ser Gln Ile Asp Pro Glu Ala Arg Thr Thr	Pro Val	Gly Leu	
225	230	235	
gtg att ggc att gag gcc gac ccg gac agg ccc gac tgg	cac	gag tgg	771
Val Ile Gly Ile Glu Ala Asp Pro Asp Arg Pro Asp	Trp His	Glu Trp	
240	245	250	
ttc tcg cgc aga ctg cag ttc tgc cag tgg acg atc	cag	gag ccg	819
Phe Ser Arg Arg Leu Gln Phe Cys Gln Trp Thr Ile	Gln Ala Lys Pro		
255	260	265	270
gga cac ccg ctg ctg cgc gag ctg atc atc cgg atc	gtg	gag gag acg	867
Gly His Pro Leu Leu Arg Glu Leu Ile Ile Arg Ile	Val Glu Glu Thr		
275	280	285	
ttc cgc aaa cag cac atg ggc gtt ttg aaa aga gtg	gaa	ggc aag gac	915
Phe Arg Lys Gln His Met Gly Val Leu Lys Arg Val	Glu Gly Lys Asp		
290	295	300	
tcg ggc gca gat atc atg cag tgg aca gga ccg	ggg	ata ttt aca gac	963
Ser Gly Ala Asp Ile Met Gln Trp Thr Gly Pro Gly	Ile Phe Thr Asp		
305	310	315	
act ctg ttt gat tat ctg aac aat gtg gcg	agg	gac ggc aag ttg ggc	1011
Thr Leu Phe Asp Tyr Leu Asn Asn Val Ala Ser	Asp Gly Lys Leu Gly		
320	325	330	
gac ggg tac ggc gtg ggg tcg ttg tat tgg cgc	aag	cac ggc aaa tat	1059
Asp Gly Tyr Gly Val Gly Ser Leu Tyr Trp Arg Lys	His Gly Lys Tyr		
335	340	345	350
aag ctg aaa aag aca gaa att aac aag aat aac	gag	cca ttg cat tct	1107
Lys Leu Lys Lys Thr Glu Ile Asn Lys Asn Asn	Glu Pro Leu His Ser		
355	360	365	
gag gac cag ctt atc aac tgg agg tcg ctg acc	aac	atg gac aag cca	1155
Glut Asp Gln Leu Ile Asn Trp Arg Ser Leu Thr Asn	Met Asp Lys Pro		
370	375	380	
aag atc atg ggg gac gta atg gtg tta cca atc	acg	ttt agt ccg	1203
Lys Ile Met Gly Asp Val Met Val Leu Pro Ile	Thr Ser Phe Ser Pro		
385	390	395	
aac gtg ggg cac atg ggc tca aag agc	agc	tca gat agg ctg gca ttt	1251
Asn Val Gly His Met Gly Ser Lys Ser Ser Asp	Arg Leu Ala Phe		
400	405	410	
gtg gag cat tta ttt tct ggc agc tgg aag cca	aaa aac	aaa tagaaaa	1300
Val Glu His Leu Phe Ser Gly Ser Trp Lys Pro	Lys Asn Lys		
415	420	425	
aataaataat tagctgcait ttagataatt ctcatgagca	ggcacagaac	g	1351
<210> 2			
<211> 428			
<212> PRT			
<213> Hansenula polymorpha			
<400> 2			
Met Val Tyr Phe Leu Asn Phe Met Ser Ile Thr Asn Val Pro Val Leu			
1 5 10 15			
Lys Arg Ala Arg Leu Tyr Met Ala Thr Asn Arg Arg Leu Val Val Val			
20 25 30			
Leu Val Val Leu Leu Tyr Trp Val Val Gln Asn Val Trp Thr Trp Ser			
35 40 45			
Pro Gly Thr Arg Asp Leu Ala Gln Val Asp Ala Lys Ile Glu Ala Glu			
50 55 60			

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Leu Asn Ser Asn Leu His Thr Phe Gly Ala His Leu Arg His Leu Asn
 65 70 75 80
 Arg Leu Pro Ala Glu Ser Ala Thr Leu Arg Glu Lys Leu Thr Phe Tyr
 85 90 95
 Phe Pro Tyr Tyr Pro Glu Lys Pro Val Pro Asn Gln Ile Trp Gln Thr
 100 105 110
 Trp Lys Val Asp Leu Glu Asp Asp Asn Phe Pro Lys Gln Tyr Arg Arg
 115 120 125
 Phe Gln Lys Thr Trp Val Glu Lys Asn Pro Asp Tyr Val Tyr His Leu
 130 135 140
 Ile Pro Asp Ser Val Ile Glu Asp Phe Val Ala Ser Leu Tyr Ala Asn
 145 150 155 160
 Val Pro Glu Val Val Arg Ala Tyr Gln Leu Leu Pro Lys Asn Ile Met
 165 170 175
 Lys Ala Asp Phe Phe Arg Tyr Leu Val Ile Tyr Ala Arg Gly Gly Thr
 180 185 190
 Tyr Ser Asp Met Asp Thr Val Cys Leu Lys Pro Ile Lys Asp Trp Ala
 195 200 205
 Thr Phe Asp Arg Asp Leu Ile His Ala Ala Asp Asn Lys Ala Asp Leu
 210 215 220
 Ser Gln Ile Asp Pro Glu Ala Arg Thr Thr Pro Val Gly Leu Val Ile
 225 230 235 240
 Gly Ile Glu Ala Asp Pro Asp Arg Pro Asp Trp His Glu Trp Phe Ser
 245 250 255
 Arg Arg Leu Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro Gly His
 260 265 270
 Pro Leu Leu Arg Glu Leu Ile Ile Arg Ile Val Glu Glu Thr Phe Arg
 275 280 285
 Lys Gln His Met Gly Val Leu Lys Arg Val Glu Gly Lys Asp Ser Gly
 290 295 300
 Ala Asp Ile Met Gln Trp Thr Gly Pro Gly Ile Phe Thr Asp Thr Leu
 305 310 315 320
 Phe Asp Tyr Leu Asn Asn Val Ala Ser Asp Gly Lys Leu Gly Asp Gly
 325 330 335
 Tyr Gly Val Gly Ser Leu Tyr Trp Arg Lys His Gly Lys Tyr Lys Leu
 340 345 350
 Lys Lys Thr Glu Ile Asn Lys Asn Asn Glu Pro Leu His Ser Glu Asp
 355 360 365
 Gln Leu Ile Asn Trp Arg Ser Leu Thr Asn Met Asp Lys Pro Lys Ile
 370 375 380
 Met Gly Asp Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro Asn Val
 385 390 395 400
 Gly His Met Gly Ser Lys Ser Ser Ser Asp Arg Leu Ala Phe Val Glu
 405 410 415
 His Leu Phe Ser Gly Ser Trp Lys Pro Lys Asn Lys
 420 425

<210> 3
 <211> 36
 <212> DNA
 <213> Artificial Sequence

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<220> primer 168Not-N
 <223>

<400> 3
 aaggaaaaaa gcggccgcccgttgaagaatggtgtat

36

<210> 4
 <211> 39
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer 168Not-C

<400> 4
 ttttcctttt gcggccgcccgttctgtgcctgtcatgtat

39

<210> 5
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer UNfor

<400> 5
 ggatccccgg gtaccgagct

20

<210> 6
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer UNrev

<400> 6
 caccggtagc taatgatccc

20

<210> 7
 <211> 20
 <212> DNA
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<220>
 <223> primer UCfor

<400> 7
 cgaacatcca agtgggccga

20

<210> 8
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer UCrev

<400> 8
 ctggcgaaag gggatgtgc

20

<210> 9
 <211> 20

PCTA9407-2(한국생명공학연구원)

<212> DNA
<213> Artificial Sequence

<220>
<223> primer 168Nfor

<400> 9
ggcggatatg gggcttcgcc

20

<210> 10
<211> 40
<212> DNA
<213> Artificial Sequence

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<400> 10
agctcggtac cggggatcc cggtccagg ctccacgtcc

40

<210> 11
<211> 40
<212> DNA
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<220>
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<400> 11
gcacatcccc cttcgccag ccgatcacga gcttcagtcc

40

<210> 12
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> primer 168Crev

<400> 12
cgtcgccgg gcccagttcg

20

<210> 13
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> primer for the amplification of alpha1,2-manosidase in
Aspergillus saitoi

<400> 13
ggggaaatca aaaaaatggt ggtttcagc aaa

33

<210> 14
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> primer for the amplification of alpha1,2-manosidase in
Aspergillus saitoi

<400> 14

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gggccatggt cacaattcat catgcgcata gtcaggaaca tcgtatgggt atgtactact	60
caccggcac	69